

US 09/306,333 Jan Vijg response May 5, 2005

2

Amendments to the claims

1. 2, 3, (Canceled)

4. (Currently amended) Test kits for enabling BRCA1 gene testing comprising a combination of ~~the primer pairs listed in Table 4 under "PRIMER SEQUENCES"~~ column PCR primer pairs SEQ ID NOS: 47 and 48, 49 and 50, 51 and 52, 53 and 54, 55 and 56, 57 and 58, 59 and 60, 61 and 62, 63 and 64, 65 and 66, 67 and 68, 69 and 70, 71 and 72, 73 and 74, 75 and 76, 77 and 78, 79 and 80, 81 and 82, 83 and 84, 85 and 86, 87 and 88, 89 and 90, 91 and 92, 93 and 94, 95 and 96, 97 and 98, 99 and 100, 101 and 102, 103 and 104, 105 and 106, 107 and 108, 109 and 110, 111 and 112, 113 and 114, 115 and 116, 117 and 118, 119 and 120 mixed in about 20mM of Tris-HCL, 50mMKCL, 25pM of dNTP and 5% formamide.
5. (Currently Amended) ~~The test kits of claim 4 wherein the gel or gel material is provided with about 20-65% of UF (urea and formamide).~~ The test kits of claim 4 wherein a gel or gel material is also provided comprising acrylamide and bisacrylamide in a ratio of about 37.5 to 1, in a buffer.
6. (Currently Amended) ~~Test kits for enabling hMLH1 gene testing comprising the short PCR primer pairs listed in Table 3 mixed in about 20mM of Tris-HCL, 50mM KCL, 25pM of dNTP and 5% formamide.~~ The test kits of claim 5 wherein a gel or gel material is provided with about 20-65% of UF (urea and formamide).

US 09/306,333 Jan Vijg response May 5, 2005

3

7. (Withdrawn) Test kits for enabling hMLH1 gene testing comprising the short PCR primer pairs listed in Table 3 mixed in about 20mM of Tris-HCl, 50mM KCL, 25μM of dNTP and 5% formamide.
8. (Withdrawn) The test kits of claim 7 wherein a gel is also provided comprising acrylamide and bisacrylamide in a ratio of about 37.5 to 1, in a buffer. —
9. (Withdrawn) The test kits of claim 8 wherein the gel is provided with about 25-75% of UF (urea and formamide).—
10. (Currently amended) A method of detecting mutations in a BRCA1 gene with the aid of PCR primers for enabling amplifying the entire coding sequence of the BRCA1 genes that comprises;
- (a) amplifying a test sample containing nucleotide sequences by long distance multiplex PCR, using primer sequence pairs SEQ ID NOS: 33 and 34, 35 and 36, 37 and 38, 39 and 40, 41 and 42, 43 and 44, 45 and 46 to produce a first set of 7 PCR amplification products with exon fragments numbered 1-3, 14-17, 18-20 and 21-24, including exons 1 through 3, 5 through 9, 10 and 11, 12 and 13, 14 through 17, 18 through 20, and 21 through 24, respectively;
- (b) ~~subjecting the this~~ first set of PCR amplification products to short distance multiplex PCR ~~to produce a second set of amplification products with (1) exon fragments numbered 11.1 through 11.6 using primer sequence pairs SEQ ID NOS: 47 and 48, 77 and 78, respectively, and (II) exon fragments numbered 2 through 10 and 12 through 24, using primer sequence pairs SEQ ID NOS. 79 and 80 through 119 and 120 to produce 37 short PCR products including/encompassing exons 2 through 24 of the BRCA1 gene;~~

US 09/306,333 Jan Vijg response May 5, 2005

4

(c) using GC clamp sequences attached to primers during said short distance multiplex PCR SEQ ID NOS: 27, 28, 29, 30, 31, and 32 attached to one or both sequences of each short multiplex PCR primer pair; and

(d) subjecting the short PCR second set of amplification products to two-dimensional gel electrophoresis to produce a characteristic spot pattern for specific mutations in the BRCA1 gene.

11. (Currently Amended) The method of claim 10 wherein gels and buffer materials are used so as to enable combined mixtures of multiple groups of BRCA1 genes to be subjected to the electrophoresis together.

12. (Withdrawn) The method of claim 10 wherein said exon fragment 11.1 is the first of a fragment split 16 times to produce exon fragments numbered 11.1 through 11.16 before said short distance multiplex PCR.

13. (Currently Amended) The method of claim 10 wherein ~~the primers for said 11.1, 11.2, 11.4, 11.6, 11.9, 11.10, 11.12, 11.14, 11.16, 12.18, and 22.24 and 25 are each clamped by a pair of GC clamps~~ PCR products generated are clamped by GC clamps as a result of attaching GC clamp SEQ ID NO: 27 to primers SEQ ID NOS: 64, 76, 82, 83, 85, 93, 95, 97, 99, 105, 108, 110, 111, 113, 115, and 118; GC clamp SEQ ID NO: 28 to primer SEQ ID NO: 120; and attaching GC clamp SEQ ID NO. 29 to primers SEQ ID NOS: 47, 49, 52, 54, 55, 57, 59, 62, 66, 67, 70, 71, 73, 77, 79, 87, 90, 91, 101, and 104.

14. (Currently Amended) The method of claim 10 wherein ~~the primers for said exon fragments numbered 6.10, 11.3, 11.7, 11.8, 11.11, 11.13, 11.16, and 19.21 are each clamped by a single GC clamp~~ PCR products generated are clamped by a pair of GC

US 09/306,333 Jan Vijg response May 5, 2005

5

clamps as a result of attaching GC clamp SEQ ID NO: 30 to primer SEQ ID NOS: 48, 58, 63, 78, 96, 100, 102, 103, 116, and 119; attaching GC clamp SEQ ID NO: 31 to primers SEQ ID NOS: 50, 53, 65, 69, 74, 80, 81, 84, 107, and 117; and attaching GC clamp SEQ ID NO: 32 to primers SEQ ID NOS: 106 and 56.

Claims 15, 16, and 17 cancelled.